FINAL TECHNICAL REPORT
AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
GRANT NO. F49620-99-1-0206
EFFECTS OF JP8 ON NEURAL STRUCTURE AND FUNCTION

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2. OBJECTIVES: To determine the effects of JP8 exposure on the levels of biogenic amines and amino acid neurotransmitters in specific brain regions of rats.

3. EXPERIMENTAL DESIGN/STRATEGY - The levels of each biogenic amine neurotransmitter and its major metabolite and each amino acid neurotransmitter were determined in each of seven brain regions in rats exposed to 1000 mg/m^3 JP8+100 for 7, 14, 21 and 28 days (six rats per group). Sham controls (12 rats) and a non-treatment group (12 rats) were included in the experimental design. If major neuronal damage has taken place we anticipate changes will be seen in the levels of specific neurotransmitters in affected brain regions. Those regions showing changes will be the focus of future micropunch sampling of specific brain nuclei.

4. STATUS OF EFFORT -

a. Methodology - Using high performance liquid chromatography with electrochemical detection (hplc/ec) we had previously established to method to determine biogenic amine neurotransmitters (norepinephrine, dopamine, epinephrine and serotonin) and their major metabolites (homovanillic acid and 5-hydroxyindoleacetic acid). We have more recently acquired the equipment required for analysis of amino acid neurotransmitters (gamma-aminobutyric acid, glutamic and aspartic acids and glycine). This method has recently been established in our laboratory. Amino acids must be derivatized with ortho-pthalaldehyde prior to chromatography and we have automated this process. Each brain sample thus required two chromatographic analyses. For these analyses, a given brain sample if homogenized in dilute perchloric acid, centrifuged and aliquots of the supernatants are injected in to the hplc instrument. Protein is determined on the precipitated protein and neurotransmitter levels can thus be expressed per my of protein.

b. Analyses - We have now received brain regions from rats exposed daily (except Saturdays and Sundays) 1000 mg/m^3 of JP8+100 for one, two, three and four weeks. Brains were removed at the University of Arizona and the following regions were dissected according to the standard Glowinski method: total cerebral cortex, hippocampus, striatum, brain stem, midbrain, hypothalamus and cerebellum. For each experiment, we received samples from 12 exposed and 6 sham control rats. The total number of chromatographic analyses will be 1008. We have completed the analysis of biogenic amines for the one and two week exposure groups and are currently analyzing the three and four week exposure groups. When these analyses are completed, we will begin the analysis of amino acid neurotransmitters. At that juncture we will know what the behavioral effects of JP8 exposure were and will use micropunch techniques to sample the affected brain region(s).

5. ACCOMPLISHMENTS/NEW FINDINGS

We have completed all analyses; the resulting data is summarized in the tables included in this report. All statistically significant differences are indicated by a bold typeface. The results indicate the great importance of including sham controls in all JP8 studies. The most significant effect was the increase of hippocampal DOPAC during the exposure period, indicative of

increased dopamine release and turnover. We conclude that with this significant exception, JP8 exposure at this level caused no global alterations in neurotransmitter levels.

Biogenic Amine Levels in Cerebral Cortex

	Norepinephrine	DOPAC	dopamine	5-HIAA	HVA	Serotonin
no treatment	2.88 ± 0.76	0.841 ± 0.141	5.18 ± 1.13	2.11 ± 0.22	0.333 ± 0.073**	4.34 ± 0.50
sham exposed	2.41 ± 0.35	1.00 ± 0.17	5.04 ± 1.09	2.28 ± 0.25	0.242 ± 0.049	4.21 ± 0.68
1 week exposed	2.71 ± 0.81	0.792 ± 0.259	5.12 ± 1.13	2.19 ± 0.22	0.314 ± 0.086	3.98 ± 0.49
2 week exposed	3.19 ± 0.62	0.778 ± 0.230	4.91 ± 1.23	2.14 ± 0.45	0.338 ± 0.083	3.99 ± 0.65
3 week exposed	2.30 ± 0.17	1.09 ± 0.35	6.11 ± 1.28	2.37 ± 0.41	0.247 ± 0.056	4.53 ± 0.61
4 week exposed	**1.89 ± 033***	0.924 ± 0.277	4.72 ± 1.26	2.18 ± 0.28	0.259 ± 0.047	3.87 ± 0.44

* $p<0.05$ from no treatment but not different from any other group except 2 week exposed
** $p<0.05$ from sham but not different from any other group

Biogenic Amine Levels in Cerebellum

	Norepinephrine	DOPAC	dopamine	5-HIAA	HVA	Serotonin
no treatment	1.18 ± 0.13	0.065 ± 0.017	0.426 ± 0.119	0.669 ± 0.107	0.093 ± 0.026	0.807 ± 0.133
sham exposed	1.08 ± 0.18	0.065 ± 0.023	0.393 ± 0.127	0.634 ± 0.148	0.075 ± 0.010	± 0.112*0.638*
1 week exposed	**1.42 ± 0.16***	0.060 ± 0.009	0.430 ± 0.064	0.711 ± 0.078	0.088 ± 0.011	0.861 ± 0.156
2 week exposed	1.18 ± 0.13	0.051 ± 0.013	0.350 ± 0.055	0.658 ± 0.051	0.079 ± 0.010	0.731 ± 0.220
3 week exposed	1.13 ± 0.15	0.060 ± 0.031	0.361 ± 0.164	0.643 ± 0.144	0.069 ± 0.015	0.651 ± 0.101
4 week exposed	1.31 ± 0.20	0.059 ± 0.012	0.309 ± 0.067	0.883 ± 0.286	0.073 ± 0.009	0.719 ± 0.042

*$p<0.01$ from sham but $p< 0.05$ from no treatment
**$p< 0.05$ from no treatment an 1 week exposed but not different from any other group

Biogenic Amine Levels in Brain Stem

	Norepinephrine	DOPAC	dopamine	5-HIAA	HVA	Serotonin
no treatment	3.09 ± 0.39	0.104 ± 0.030	0.563 ± 0.086	2.49 ± 0.39	0.126 ± 0.021	4.58 ± 0.77
sham exposed	2.86 ± 0.27	0.119 ± 0.031	0.559 ± 0.082	2.70 ± 0.33	0.115 ± 0.020	4.54 ± 0.55
1 week exposed	2.96 ± 0.29	0.135 ± 0.040	0.706 ± 0.198	2.36 ± 0.29	0.133 ± 0.019	4.16 ± 0.57
2 week exposed	3.29 ± 0.43	0.125 ± 0.024	0.647 ± 0.090	2.73 ± 0.43	0.136 ± 0.018	4.97 ± 0.56
3 week exposed	2.85 ± 0.58	0.145 ± 0.034	0.611 ± 0.122	2.91 ± 0.42	0.123 ± 0.027	4.61 ± 0.96
4 week exposed	3.27 ± 0.33	0.129 ± 0.023	0.616 ± 0.043	2.89 ± 084	0.127 ± 0.022	5.04 ± 0.77

Biogenic Amine Levels in Striatum

	Norepinephrine	DOPAC	dopamine	5-HIAA	HVA	Serotonin
no treatment	1.63 ± 0.37	4.87 ± 1.49	29.7 ± 7.3	3.40 ± 0.51	1.53 ± 0.40	3.59 ± 0.64
sham exposed	1.53 ± 0.23	5.53 ± 1.66	27.3 ± 6.6	3.73 ± 0.62	1.53 ± 0.31	3.94 ± 0.64
1 week exposed	1.51 ± 0.30	4.70 ± 1.01	28.9 ± 5.4	3.29 ± 0.62	1.55 ± 0.27	3.59 ± 0.76
2 week exposed	2.08 ± 0.73	5.57 ± 1.76	32.7 ± 3.9	3.94 ± 0.58	1.66 ± 0.31	4.15 ± 0.55
3 week exposed	1.40 ± 0.31	6.59 ± 2.60	27.4 ± 6.8	3.68 ± 0.85	1.76 ± 0.55	3.62 ± 0.65
4 week exposed	1.41 ± 0.22	5.39 ± 1.08	26.3 ± 5.0	3.60 ± 0.40	1.51 ± 0.21	3.57 ± 050

Biogenic Amine Levels in Midbrain

	Norepinephrine	DOPAC	dopamine	5-HIAA	HVA	Serotonin
no treatment	2.32 ± 0.49	0.331 ± 0.109	1.96 ± 0.40	3.73 ± 0.65*	0.184 ± 0.035	5.48 ± 1.03
sham exposed	2.61 ± 0.17	0.345 ± 0.079	1.77 ± 0.41	4.68 ± 0.45	0.188 ± 0.031	6.33 ± 052
1 week exposed	2.53 ± 0.37	0.353 ± 0.141	2.11 ± 0.65	4.12 ± 0.74	0.209 ± 0.072	5.13 ± 0.49
2 week exposed	2.54 ± 0.41	0.390 ± 0.069	2.42 ± 0.42	4.28 ± 0.77	0.214 ± 0.032	6.30 ± 0.49
3 week exposed	2.62 ± 0.54	0.419 ± 0.117	2.17 ± 0.35	4.56 ± 1.28	0.215 ± 0.058	6.54 ± 1.30
4 week exposed	2.54 ± 0.15	0.374 ± 0.064	1.80 ± 0.19	4.48 ± 0.53	0.205 ± 0.027	6.02 ± 0.66

*p< 0.05 from sham but not different from any other group

Biogenic Amine Levels in Hypothalamus

	Norepinephrine	DOPAC	dopamine	5-HIAA	HVA	Serotonin
no treatment	3.95 ± 0.79	0.321 ± 0.112**	1.45 ± 0.38	2.45 ± 0.58	0.128 ± 0.046#	3.32 ± 0.84
sham exposed	5.38 ± 1.46	0.538 ± 0.175***	1.88 ± 0.59	3.68 ±1.29	0.159 ± 0.045	4.32 ± 1.01
1 week exposed	4.19 ± 0.83	0.347 ± 0.109	1.70 ± 0.51	2.47 ± 0.37	0.142 ± 0.045	3.35 ± 0.39
2 week exposed	4.45 ± 0.53	0.317 ± 0.102	1.55 ± 0.22	2.65 ± 0.42	0.120 ± 0.039	3.73 ± 0.43
3 week exposed	5.60 ± 1.34	0.496 ± 0.146	1.84 ± 0.54	3.22 ± 1.02	0.155 ± 0.037	4.32 ± 1.12
4 week exposed	**6.24 ± 1.14***	0.648 ± 0.139	2.28 ± 0.44	3.71 ± 0.86	0.189 ± 0.045	4.82 ± 1.49

*p< 0.01 from no treatment but not different from any other group
**p< 0.01 from sham and 4 week exposed
***p< 0.05 from no treatment, 1 week, and 2 week exposed
# p< 0.05 from sham and 4 week exposed

Biogenic Amine Levels in Hippocampus

	Norepinephrine	DOPAC	dopamine	5-HIAA	HVA	Serotonin
no treatment	1.48 ± 0.21	0.107 ± 0.026	0.446 ± 0.132	2.32 ± 0.41	0.071 ± 0.017	3.31 ± 1.51
sham exposed	1.79 ± 0.29*	0.104 ± 0.035	0.538 ± 0.120	2.77 ± 0.41	0.072 ± 0.017	3.70 ± 1.64
1 week exposed	1.31 ± 0.21	0.083 ± 0.023	0.401 ± 0.105	2.20 ± 0.26	0.066 ± 0.019	3.06 ± 1.40
2 week exposed	1.78 ± 0.25	0.110 ± 0.026	0.512 ± 0.142	2.42 ± 0.54	0.074 ± 0.019	3.35 ± 1.40
3 week exposed	**1.86 ± 0.15****	0.118 ± 0.026	0.517 ± 0.040	3.13 ± 0.24	0.085 ± 0.004	3.92 ± 1.37
4 week exposed	1.77 ± 0.31	**± 0.043*0.147****	0.505 ± 0.348	2.69 ± 0.50	0.077 ± 0.017	4.05 ± 2.35

*p< 0.05 from no treatment but p< 0.01 from 1 week exposed
**p< 0.05 from no treatment but not different from any other group
***p< 0.05 from no treatment and sham

All data was expressed as ng neurotransmitter/mg of protein. Results were expressed as mean ± SD. All groups were tested for normality and equal variance. If those tests passed, one way ANOVA was used to determine significance followed by Dunnett's test when appropriate. If normality test failed, Kruskal-Wallis one way analysis of variance on ranks was used to determine significance followed by Dunn's test when appropriate.

Amino Acid Levels in Cerebral Cortex

	Glutamate	GABA	Glycine	Glutamine	Serine
no treatment	65.8 ± 6.21	56.4 ± 10.0	20.4 ± 1.69	21.0 ± 4.14	15.4 ± 1.13
sham exposed	66.2 ± 5.41	54.0 ± 8.61	19.8 ± 2.20	20.7 ± 3.39	14.9 ± 1.28
1 week exposed	68.1 ± 7.61	57.8 ± 11.3	**25.0 ± 2.67***	20.8 ± 4.71	**18.1 ± 1.61****
2 week exposed	68.3 ± 10.1	46.7 ± 13.2	21.1 ± 2.79	17.9 ± 4.01	15.2 ± 2.11
3 week exposed	63.1 ± 13.3	52.6 ± 9.08	21.4 ± 3.03	20.2 ± 3.67	15.9 ± 2.20
4 week exposed	63.3 ± 7.59	48.4 ± 8.83	19.6 ± 1.43	17.7 ± 3.52	14.2 ± 1.44

* p< 0.01 from no treatment control and sham
** p< 0.01 from no treatment control and sham

Amino Acid Levels in Cerebellum

	Glutamate	GABA	Glycine	Glutamine	Serine
no treatment	63.5 ± 10.5	40.0 ± 8.62	21.5 ± 3.93	21.0 ± 4.15	13.6 ± 1.56
sham exposed	64.8 ± 7.70	37.7 ± 5.70	21.6 ± 2.25	19.8 ± 3.05	13.8 ± 1.55
1 week exposed	68.3 ± 6.02	43.1 ± 6.99	19.8 ± 1.27	22.3 ± 3.26	14.0 ± 1.20
2 week exposed	64.9 ± 4.43	39.8 ± 6.55	18.9 ± 1.13	21.6 ± 3.71	12.9 ± 2.64
3 week exposed	67.0 ± 6.93	42.6 ± 5.62	19.7 ± 1.46	22.8 ± 3.91	13.0 ± 1.32
4 week exposed	71.4 ± 6.45	**46.0 ± 4.58***	23.1 ± 2.78	22.0 ± 3.89	14.0 ± 1.16

Amino Acid Levels in Brain Stem

	Glutamate	GABA	Glycine	Glutamine	Serine
no treatment	44.4 ± 7.50	31.3 ± 3.63	42.0 ± 5.20	12.4 ± 3.36	12.2 ± 3.56
sham exposed	45.4 ± 7.31	31.3 ± 7.96	42.4 ± 4.12	12.4 ± 2.73	12.4 ± 5.49
1 week exposed	39.6 ± 6.32	27.4 ± 7.34	39.8 ± 4.80	10.6 ± 2.46	11.6 ± 2.33
2 week exposed	51.3 ± 6.40	34.3 ± 4.47	45.7 ± 4.32	14.5 ± 1.74	11.2 ± 1.79
3 week exposed	47.4 ± 9.33	33.6 ± 8.18	43.6 ± 7.33	13.2 ± 2.84	10.1 ± 1.60
4 week exposed	48.3 ± 8.67	30.8 ± 3.86	38.4 ± 8.48	12.6 ± 2.82	10.5 ± 2.04

Amino Acid Levels in Striatum

	Glutamate	GABA	Glycine	Glutamine	Serine
no treatment	54.5 ± 10.1	41.1 ± 9.43	38.9 ± 8.68	19.8 ± 5.34	17.8 ± 2.02
sham exposed	66.0 ± 10.3	59.1 ± 11.6*	29.4 ± 5.54	18.2 ± 3.35	16.3 ± 2.77
1 week exposed	67.2 ± 15.8	52.1 ± 14.2	30.7 ± 5.73	18.3 ± 4.58	16.0 ± 2.52
2 week exposed	58.0 ± 13.8	45.2 ± 13.7	34.3 ± 10.2	15.7 ± 3.81	17.4 ±3.33
3 week exposed	60.2 ± 8.81	**42.4 ± 5.18****	44.3 ± 16.7	15.4 ± 2.46	20.6 ± 6.13
4 week exposed	61.9 ± 9.98	50.1 ± 8.06	31.1 ± 6.63	16.2 ± 2.53	17.1 ± 3.28

* $p < 0.01$ from no treatment control

** $p < 0.05$ sham but not different from no treatment

Amino Acid Levels in Midbrain

	Glutamate	GABA	Glycine	Glutamine	Serine
no treatment	61.0 ± 6.23	84.1 ± 9.38	31.3 ± 3.40	18.6 ± 2.45	11.2 ± 1.84
sham exposed	62.2 ± 10.9	87.9 ± 20.3	33.9 ± 3.33	19.4 ± 3.88	12.5 ± 2.79
1 week exposed	57.4 ± 17.0	75.7 ± 20.5	28.1 ± 4.26	17.5 ± 4.70	10.9 ± 2.87
2 week exposed	58.0 ± 9.63	80.3 ± 16.2	31.1 ± 4.53	18.2 ± 3.04	10.3 ± 1.61
3 week exposed	62.9 ± 8.16	87.8 ± 17.7	32.7 ± 7.83	20.3 ± 3.32	11.5 ± 2.83
4 week exposed	58.1 ± 3.09	78.8 ± 8.18	30.4 ± 8.17	17.3 ± 2.51	12.0 ± 3.35

Amino Acid Levels in Hypothalamus

	Glutamate	GABA	Glycine	Glutamine	Serine
no treatment	39.1 ± 9.31	47.2 ± 6.61	29.2 ± 4.64	12.1 ± 2.86	12.6 ± 3.09
sham exposed	47.4 ± 4.79	51.3 ± 7.59	27.5 ± 5.61	14.0 ± 1.26	11.1 ± 2.05
1 week exposed	39.6 ± 8.10	51.7 ± 14.2	27.9 ± 4.81	12.0 ± 2.87	13.0 ± 1.71
2 week exposed	49.5 ± 10.3	43.2 ± 13.6	**20.9 ± 4.54***	13.7 ± 3.21	**8.67 ± 1.81*****
3 week exposed	44.7 ± 6.26	47.8 ± 11.1	25.1 ± 5.85	13.5 ± 2.29	11.6 ± 3.02
4 week exposed	46.0 ± 8.41	56.7 ± 7.61	**20.9 ± 3.13****	14.3 ± 2.01	**9.00 ± 1.95******

* $p < 0.05$ from no treatment but not different from sham
** $p < 0.05$ from no treatment but not different from sham
*** $p < 0.05$ from no treatment but not different from sham
**** $p < 0.019$ from no treatment but not different from sham

Amino Acid Levels in Hippocampus

	Glutamate	GABA	Glycine	Glutamine	Serine
no treatment	75.2 ± 5.96	46.0 ± 10.3	22.4 ± 2.41	18.7 ± 3.57	13.4 ± 1.12
3 week sham	75.3 ± 8.29	53.3 ± 12.8	23.8 ± 4.90	20.2 ± 3.64	14.3 ± 2.34
1 week exposed	73.2 ± 6.44	43.6 ± 4.21	21.3 ± 3.49	15.9 ± 3.83	12.8 ± 1.58
2 week exposed	72.0 ± 6.98	39.3 ±9.89	23.1 ± 4.27	16.8 ± 2.89	13.6 ± 1.35
3 week exposed	67.9 ± 8.71	**36.2 ± 8.07***	24.5 ± 4.82	15.6 ± 3.70	13.9 ± 1.32
4 week exposed	75.5 ± 3.88	46.8 ± 6.07	21.6 ± 1.84	20.0 ± 3.16	13.2 ± 0.71

* $p < 0.05$ from sham but not different from no treatment control

All data was expressed as nmol neurotransmitter/mg of protein. Results were expressed as mean ± SD. All groups were tested for normality and equal variance. If those tests passed, one way

ANOVA was used to determine significance followed by Dunnett's test when appropriate. If normality test failed, Kruskal-Wallis one way analysis of variance on ranks was used to determine significance followed by Dunn's test when appropriate.

CAVEAT

While the results from the micropunch experiment indicate only two modest effects of JP8 exposure, these data may have been compromised as the rats were sacrificed by injection of anesthetic drugs, contradicting our agreed upon protocol. We have no way of knowing whether any JP8 effects were masked by these drugs. In addition, no sham-exposed controls were included in the experiment, again, contradicting previous agreed upon protocols; this omission further complicates interpretation of our data.

FUTURE

Professor Vincente Montero has indicated his interest in continuing the micropunch experiments, furnishing samples to Dr. Frank Witzmann for 2D gel analysis.

6. PERSONNEL SUPPORTED

Frank L. Siegel, Ph.D., Principal Investigator
Lynda S. Wright, M.S., Senior Researcher

7. NEW PUBLICATIONS

Witzmann, F. A., Bauer, M. D., Fieno, A. M., Grant, R. A., Keough, T. W., Kornguth, S. E., Lacey, M. P., Siegel, F. L., Sun, Y., Wright, L. S., Young, R. S. and Witten, M. L. (1999) Proteomic analysis of simulated occupational jet fuel exposure in the lung. Electrophoresis **20**, 3659-3669.

8. INTERACTIONS/TRANSITIONS: We continue our interactions and collaboration with the Witten Laboratory (Arizona) and the Witzmann Laboratory (Indiana). Dr. Kornguth continues as a consultant to the Institute of Defense Analysis on biotechnology.

9. NEW DISCOVERIES, INVENTIONS OR PATENT DISCLOSURES: None

10. HONORS/AWARDS: None

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13. ABSTRACT *(Maximum 200 words)*

We have completed all analyses. The results indicate the great importance of including sham controls in all JP8 studies. THe most significant effect was the increase of hippocampal DOPAC during the exposure period, indicative of increased dopamine release and turnover. We conclude that with this significant exception, JP8 exposure at this level caused no global alterations in neurotransmitter levels.

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